Can Microwave Radiation at High Temperatures Reduce the Toxicity of Fibrous Crocidolite Asbestos?

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Exposure of animals and humans to crocidolite asbestos fibers produces fibrosis and two types of cancers: bronchogenic carcinoma and mesothelioma. It is therefore desirable to reduce toxicity of these fibers without affecting their other characteristics. In this study, commercial crocidolite asbestos fibers were radiated with microwave radiation at different temperatures. Radiated fibers and nonradiated original fibers were then studied by Mössbauer spectroscopy to quantify the amount of ferric and ferrous ions present at structurally different sites in each crocidolite sample. They were also studied for their ability to initiate the peroxidation of linoleic acid to assess the effect of radiation on this process. Results showed that microwave radiation reduced the total Fe^{2+}/Fe^{3+} ratio. This reduction produced a concomitant decrease in the ability of the radiated samples to peroxidize linoleic acid. — *Environ Health Perspect* 105(Suppl 5):1041–1044 (1997)

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Introduction

The role of iron in the toxicity of asbestos fibers has recently been reviewed (*1,2*). The ability of iron to reduce oxygen, and to decompose hydrogen peroxide and produce hydroxyl radicals, is proposed as one of the reasons for this toxicity (*3–5*).

$$Fe^{2+} \rightarrow Fe^{3+}$$
$$O_2 \rightarrow O_2^{\bullet -}$$
$$O_2^{\bullet -} + O_2^{\bullet -} \rightarrow H_2O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^-$$

It is proposed that the hydroxyl radicals produced from these reactions may attack biological macromolecules such as membrane lipids and lead to their peroxidation. The latter process may in turn generate other free radicals, as well as hydroperoxides and carbonyl end products, and amplify the damage (*6*).

Crocidolite contains iron in its ferric and ferrous forms and catalyzes the above reactions (*3–5*) and supports the peroxidation of lipids (*7,8*). Crocidolite asbestos has numerous desirable properties for technological applications, but it is also the most toxic asbestiform known. Therefore, it is desirable to reduce the toxicity of crocidolite in a manner that does not interfere with its desirable properties. In this study, we investigated the possibility of changing the oxidation state of iron in crocidolite asbestos by microwave radiation to reduce its toxicity.

Materials and Methods

Local South African commercial crocidolite was obtained from GENCOR (Griqualand Exploration and Finance, Kuruman, South Africa). The fibers were crushed by a mechanical crusher to minimize orientation effects in the Mössbauer studies. The crushed samples were 3 μm in diameter and from 5 to 10 μm in length. Some of the fibers were radiated at 300°C for 20 min by 2.3 GHz at 7.5 kW. Other samples were radiated by 2.3 GHz at 7.5 kW for 20 min under carbon dioxide and at either 110 or 165°C. *N,O*-bis(Trimethylsilyl)tri-fluoroacetamide (BSTFA) and dichloromethane were obtained from Merck (Darmstadt, Germany), pyridine from BDH (British Drug House, Poole, England), and sodium borohydride ($NaBH_4$) from Aldrich (Milwaukee, WI). Linoleic acid was purchased from Sigma (St. Louis, MO). All reagents were of analytical grade.

Mössbauer spectra were recorded in the conventional geometry at room temperature for both samples. A ^{57}Co (Rh) source at this temperature and an Austin Associates K3 linear motor (Texas) in triangular mode were used. Velocity calibrations were performed before and after each measurement using a laser interferometer system. Each spectrum and its mirror image were fitted simultaneously using the analyzing program MOSFUN (Mössbauer Data Center, University of North Carolina, Asheville, NC). This program uses least chi-square approach; the values obtained ensure full degree of confidence in the fittings (*9*).

Crocidolite samples before and after microwave radiation were also used to study the ability of the samples to initiate lipid peroxidation. Ninety milligrams of linoleic acid in 2 ml of 0.25 mM Tris buffer (pH 7.4) was incubated at 37°C for 21 hr. To study the effect of the crocidolite fibers, we added 2 mg of the fibers to this mixture to obtain a final concentration of 1 mg/ml and it was incubated for the same length of time. The preparation of fiber suspensions in buffers was easier for the original fibers than the radiated samples. However, this difficulty was observed only at the initial stages of preparation of these suspensions. Hydroperoxides and aldehydic end products were detected from the peroxidation of linoleic acid by a gas chromatography–mass spectrometry detector (GC–MSD) as reported earlier, with some modification (*10,11*). In summary, hydroperoxides were extracted into dichloromethane (10 ml), dried with anhydrous Na_2SO_4, filtered, and evaporated under nitrogen. One milliliter methanol

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Abbreviations used: BSTFA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; GC–MSD, gas chromatography–mass spectrometry detector; 9-HPODE, 9-hydroperoxy-octadecadienoic acid; 13-HPODE, 13-hydroperoxy-octadecadienoic acid.

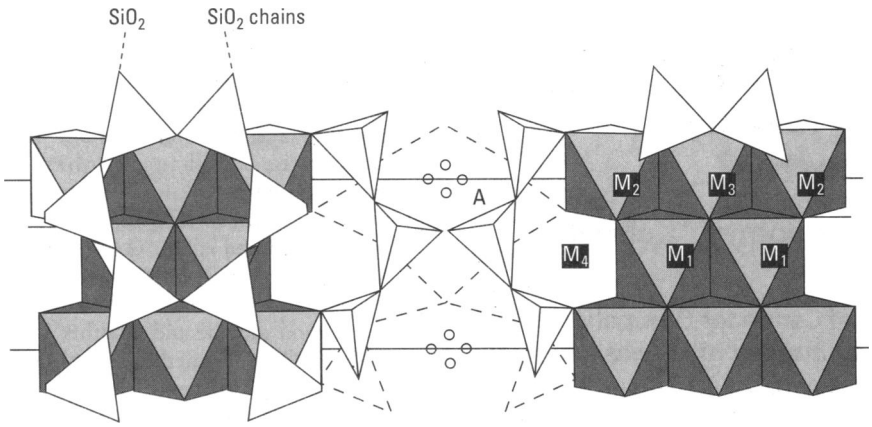

Figure 1. Schematic representation of the crystallographic sites for a unit cell of crocidolite asbestos. M_1 sites contain metal ions; A sites are often empty.

was added, followed by the addition of 1 ml $NaBH_4$ (0.793 M) solution in methanol. The samples were then incubated at room temperature for 1 hr with frequent stirring; the reaction was stopped by the addition of 2 ml water. Samples were extracted into 10 ml ether, followed by the addition of freshly prepared excess diazomethane until the reaction was complete. Excess ether was then evaporated and the samples were again extracted into dichloromethane, dried, filtered, and evaporated to 300 µl. Pyridine (50 µl) and BSTFA (50 µl) were added to form trimethylsilyl derivatives. Samples were stored at –20°C until they were analyzed. A 1-µl sample was injected into the GC–MSD.

For aldehydic end products, 1 ml of 1 M sodium borohydride was added to the incubated mixture at the end of 21 hr incubation and the samples were further incubated for 60 min at room temperature. Hydrochloric acid (1 M) was added to reduce the pH to 1 and extracted twice with 10 ml dichloromethane. The combined dichloromethane extracts were evaporated under N_2 to a residual volume of approximately 300 µl, and the residue was treated with 50 µl pyridine and 50 µl BSTFA. A 1-µl aliquot was then injected into the GC–MSD.

All hydroperoxide and aldehydic end product samples were analyzed on a Hewlett-Packard 5890 gas chromatograph (Geneva, Switzerland) coupled to a 5970 mass selective detector equipped with a HP-5MS column (30m×0.25mm id×0.25 µm film thickness) for hydroperoxides and a DB-1 capillary column (30×0.2 mm id×0.33 µm film thickness) for aldehydic end products. Significance of the results obtained (p values) were calculated using the analysis of variance test with the EpiInfo program developed by the World Health Organization and the Centers for Disease Control (*12*).

Results and Discussion

The structure of crocidolite is depicted in Figure 1 (*13*); along the c axis are double chains of tetrahedrally coordinated SiO_2 and separate ribbons containing M_1, M_2, M_3, and M_4 cationic sites. Iron ions occupy the M_1, M_2, and M_3 sites. The M_4 site accommodates alkali metal ions such as Na, K, and Ca. Figure 1 also shows the A sites, which are often vacant. On amphibole fibers, there are cleavage surfaces, which are covered with hydroxyl groups and believed to behave as electron donors (*14*).

The Mössbauer spectra of the crocidolite samples measured at room temperature before and after radiation at 300°C are represented in Figure 2. The spectra involve the superposition of quadrupole doublets corresponding to Fe at different local environments in either ferric or ferrous oxidation states. The difference in signal intensity presented on the y axis of these spectra is of no relevance because results are presented as a percent distribution of the two ionic states of iron and not their absolute concentration at different crystallographic sites in the mineral.

Table 1 shows Mössbauer parameters measured for the above two samples. Fe-site population shown in this table indicates that in the original sample, the M_1 site contained both oxidation states of iron in proportions of 25% Fe^{2+} and 22% Fe^{3+} ions. M_2 contained only Fe^{3+} (21%) and M_3 contained only Fe^{2+} (33%). Site occupation of ferric and ferrous ions agreed with results obtained with Union Internationale Contre

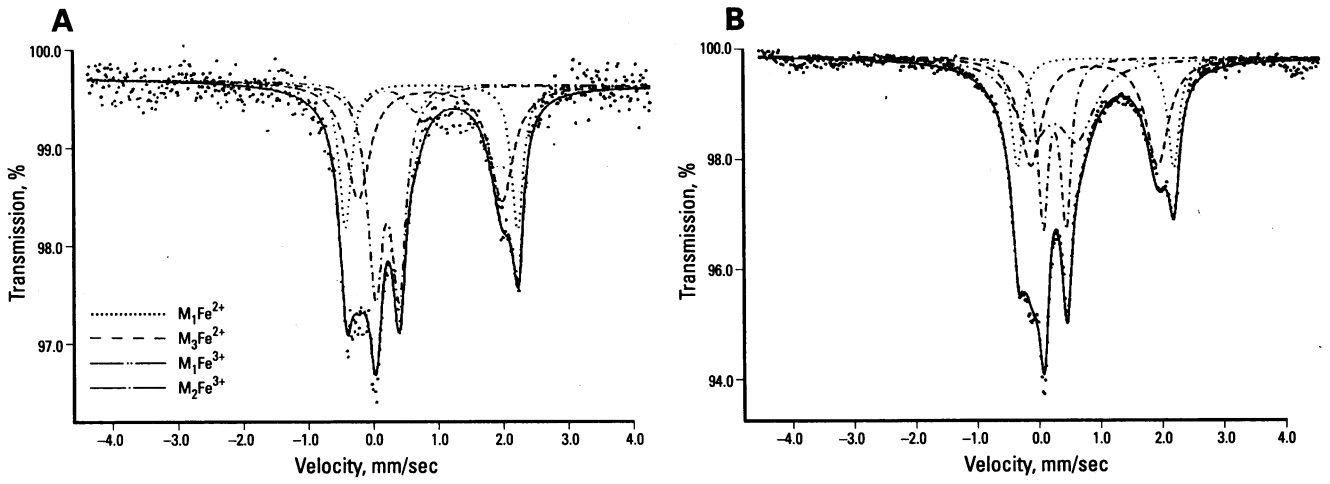

Figure 2. Mössbauer spectra of the (*A*) original and (*B*) radiated crocidolite fibers recorded at 300°K and fitted with four doublets.

Table 1. Parameters of the Mössbauer spectra recorded at room temperature for original and radiated (300°C) crocidolite samples. Isomeric shift S has been estimated relative to α-iron. The uncertainty is estimated to be ±0.02 mm/sec.

Crocidolite sample	Site designation	S, mm/sec	Δ, mm/sec	Γ, mm/sec	A, %	Fe^{2+}/Fe^{3+} ratio
Original						
	M_1Fe^{2+}	0.97	2.59	0.25	25	
	M_3Fe^{2+}	1.06	2.00	0.41	33	
Total Fe^{2+}					58	
	M_1Fe^{3+}	0.22	0.73	0.33	22	
	M_2Fe^{3+}	0.33	0.34	0.28	21	
Total Fe^{3+}					43	
						1.34
Radiated						
	M_1Fe^{2+}	0.91	2.50	0.11	16	
	M_3Fe^{2+}	0.89	2.10	0.22	31	
Total Fe^{2+}					47	
	M_1Fe^{3+}	0.23	0.75	0.34	32	
	M_2Fe^{3+}	0.24	0.37	0.10	21	
Total Fe^{3+}					53	
						0.89

Abbreviations: S, isomeric shift, which is the center of the two quadrupole doublets and is proportional to the electron density at the nucleus; Δ, quadrupole splitting and the distance between the two lines forming the quadrupole doublet; Γ, line width of the quadrupole doublets; A, the ratio of the area of the doublets to that of the total absorption area.

le Cancer crocidolite samples tested earlier but differed in their percent distribution at the corresponding sites (*13,15*). This is a common observation in mineralogy, as confirmed by other authors; the source from which crocidolite is obtained determines the content of iron and its ferrous and ferric ion distribution in the mineral (*16*). However, the results reported in this investigation may not be affected by this observation, as the changes in percent distribution of ferrous and ferric ions after microwave radiation were produced in the crocidolite samples that came from the same source as the control fibers.

It can also be seen from Table 1 that microwave radiation of the samples decreased the ferrous population from 25 to 16% and increased the ferric population from 22 to 32% at the M_1 site. However, this treatment did not affect the ferrous population at the M_3 site (31%) or the ferric population at the M_2 site (21%). The treatment, therefore, changed the ratio of Fe^{2+}/Fe^{3+} from 1.34 to 0.89 by converting the ferrous ions into ferric ions at the M_1 site.

At this point, the question arises whether these changes were due to microwave radiation or to the high temperature at which the samples were radiated. A study reported earlier (*17*) showed that when crocidolite asbestos was subjected to a temperature of 450°C for 24 hr under pure oxygen, oxy-crocidolite was produced and ferrous ions were converted into ferric ions. Mössbauer studies on these samples confirmed this conversion (*16*). Although the experiments conducted in this study do not differentiate between the role of heat treatment and microwave radiation in the conversion of ferrous to ferric ions, they show that this conversion could be achieved under milder conditions, i.e., at a temperature of 300°C and within 20 min under atmospheric oxygen.

In an attempt to obtain some clarification on the effects of heat and microwave radiation on the activity of crocidolite, some samples were radiated for 20 min at lower temperatures (110 and 165°C) and under an atmosphere of carbon dioxide. These and the two previous crocidolite samples were then tested for their ability to initiate the peroxidation of linoleic acid. The two identified hydroperoxides and the five identified carbonyl end products from the peroxidation of linoleic acid are presented in Tables 2 and 3. In the control samples containing linoleic acid with no fiber, small quantities of both 9- and 13-hydroperoxy-octadecadienoic acids (HPODE) were detected (Table 2). Of the aldehydic end products, however, only hexanal and 4-hydroxynonenal could be identified (Table 3).

The addition of the original crocidolite fibers to the incubation mixture could increase the level of two hydroperoxides by a factor of 3.40 for 9-HPODE ($p = 0.003$) and 2.47 for 13-HPODE ($p = 0.0009$). The crocidolite radiated at 300°C, on the other hand, could produce only a small nonsignificant increase ($p = 0.131$) in the 9-HPODE (1.4-fold) and a nonsignificant decrease ($p = 0.255$) in the 13-HPODE (Table 2). With aldehydic end products, the addition of the original crocidolite fibers could produce changes in the types of products observed, such as the detection of heptanal, 2-heptenal, and nonenal. It could also produce an increase in the levels of hexanal ($p = 0.0008$) and 4-hydroxynonenal ($p = 0.0005$) compared to the control samples. The addition of the samples radiated at 300°C to the reaction mixture could produce the same end products as the original crocidolite samples but at

Table 2. Two identified BSTFA derivatives of alcohols obtained from reduction of corresponding peroxides produced from peroxidation of linoleic acid in the absence (control) and presence of a final 1 mg/ml concentration of original commercial crocidolite or 1 mg/ml radiated crocidolite.

Sample	9-HPODE $\times 10^6$	13-HPODE $\times 10^6$
Control	2.68	0.63
Original	9.12	1.57
Radiated, 300°C	3.72	0.56

Values of each product were calculated as the area under the peaks obtained from mass spectra, and the results presented were calculated as the mean of three measurements. The significance of these results is stated in the text.

Table 3. Five identified BSTFA derivatives of alcohols obtained from reduction of corresponding aldehydes produced from the peroxidation of linoleic acid in the absence (control) and presence of a final 1 mg/ml concentration of original commercial crocidolite or 1 mg/ml crocidolite radiated at different temperatures.

Sample	Hexanal $\times 10^6$	Heptanal $\times 10^6$	2-Heptenal $\times 10^6$	Nonenal $\times 10^6$	4-Hydroxynonenal $\times 10^6$
Control	13.27	0.00	0.00	0.00	8.15
Original	46.14	4.95	4.51	10.31	51.29
Radiated, 300°C	16.39	1.72	2.55	1.96	29.08
Radiated, 165°C	17.81	1.81	1.97	0.00	19.56
Radiated, 110°C	15.43	1.77	1.44	0.00	14.83

Values of each product were calculated as the area under the peaks obtained from mass spectra, and the results presented were calculated as the mean of three measurements. The significance of these results is stated in the text.

less significantly different levels than control samples containing no fibers ($p = 0.28$ for hexanal, $p = 0.05$ for 4-hydroxynonenal) (Table 3). Similarly, the addition of crocidolite radiated at lower temperatures (110 and 165°C) and under an atmosphere of carbon dioxide could also produce significantly lower concentrations of these aldehydes compared to the original crocidolite fibers. These results may indicate that the microwave treatment (and perhaps not the temperature) was able to reduce the ability of crocidolite fibers to initiate lipid peroxidation and/or to decompose the preexisting lipid hydroperoxides. Mössbauer studies are presently in progress to establish the changes of ferrous and ferric ions that have occurred after crocidolite exposure to microwave radiation at 110 and 165°C.

The conversion of ferrous ions into ferric ions in crocidolite fibers, whether because of heat or microwave radiation, also indicates that this conversion produced a decrease in the ability of crocidolite fibers to initiate lipid peroxidation. Such a decrease may be an indication of a reduction in the toxicity of crocidolite samples due to the important physiological and pathological functions of some lipid peroxidation products, as stated earlier.

In a previous study (*18*), the conversion of ferric ions into ferrous ions by reduction with hydrogen produced more active fibers, which was attributed to an increase of ferrous ions occupying the M_1 sites (*19*). From these results and from those discussed in this study, it can be concluded that by changing the oxidation state of iron, especially at the M_1 site, it is possible to change the activity of the fibers. We also propose that the valency of iron is an important factor that may determine the activity of crocidolite fibers, which confirms observations made by other authors (*20,21*). Further tests are required to confirm the reduced toxicity of radiated fibers toward cells in culture in the presence of various biologically available reducing and chelating agents. These studies are necessary to investigate the behavior of oxidized iron in the M_1 site under these conditions.

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